IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF

Leonid R. PTITSYN, et al : GROUP ART UNIT: 1652

SERIAL NO: 09/886,135 : EXAMINER: Elizabeth Slobodyansky

FOR: MUTANT N-ACETYLGLUTAMATE SYNTHASE AND METHOD FOR PRODUCING L-ARGININE

DECLARATION UNDER 37 C.F.R. SECTION 1.132

ASSISTANT COMMISSIONER FOR PATENTS

WASHINGTON, D.C. 20231

SIR

Now comes Leonid R. PTITSYN who deposes and states that:

1. I am a graduate of the Moscow State University, Russia, where I majored in bioorganic chemistry, chemistry of natural and physiologically active compounds, and were I received my doctorate degree.

2. I have been employed by AGRI for 5 years as a researcher in the field of molecular biology.

3. I am named inventor of the above-identified application.

4. I have read and understood the Office Action dated June 26, 2003 and the documents cited therein.

Detailed scheme of cloning the wild type *argA* gene .

It is known that during polymerase chain reaction (PCR) used for gene's cloning some nucleotide substitutions could occur and further confirmation of gene sequence is necessary. So, the best way to obtain wild type (wt) gene is the direct isolation of the gene from the chromosome of wt strain.

In the particular case, the *E. coli* strain K12 MC1040-2 (Castilho BA et al., 1984. J. Bacteriol., 158:488-495) (VKPM B 6804) was used as a donor of wt *argA* gene.

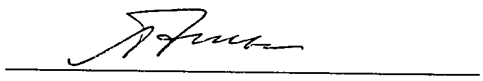
The strain MC1040-2 was transformed by the phage Mu d5005 (Groisman EA and Casadaban MJ, 1986, J. Bacteriol., 168:357-364) yielded strain MC1040-2(Mu d5005). The Mu lysate was prepared (Groisman EA and Casadaban MJ, 1986, J. Bacteriol., 168:357-364) and used for infection of *E. coli* strain K12 $argA^-$ (VKPM B-3083). The transductants were selected for Km^R and $argA^+$ phemotype. Plasmids Mu d5005-argA were purified and one of them (Mu d5005-argA-81) was used for subcloning the *argA* gene. The plasmid Mu d5005-argA-81 was digested by *Bam*HI and *Sal*I restriction enzymes and 2.02 kb *Bam*HI-*Sal*I DNA fragment carried wt *argA* gene was purified. Then DNA fragment was cloned into plasmid pUC19 yielded plasmid pUC19-ArgA (as it is written in the Example 1, section <1>). So, it was assumed that real wt *argA* gene was cloned and the sequence of the wt *argA* gene is identical to that of disclosed in the GenBank under accession number Y00492, since the same *E. coli* strain was used.

Additional proof of the above conclusion is following. The nucleotide sequence of the mutant *argA*-r13 gene (Table 2) was determined and was found to be completely identical to the wt *argA* sequence (Y00492), except the mutated region (amino acids 15 to19).

Remarks

I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of application or any patent issuing thereon.

Date: October 6, 2003



Leonid R. PTITSYN